

# CONTEXT-DEPENDENT PHOTODIMERISATION IN ISOLATED THYMINE-THYMINE STEPS IN DNA

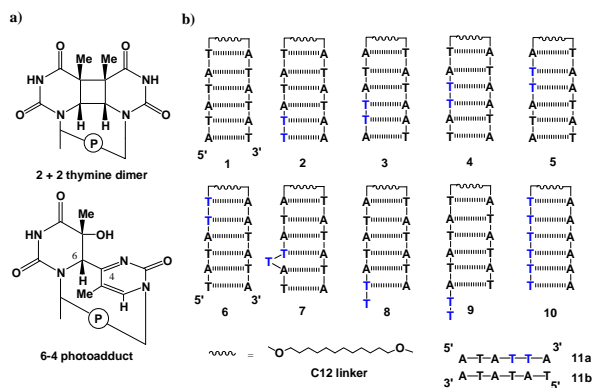
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## INTRODUCTION

Dimerization of thymine has long been recognized as a leading cause of cellular photodamage by ultraviolet light.<sup>(1)</sup> A revival of interest in T-T dimerization has been driven by advances in fast spectroscopy,<sup>(2)</sup> studies of ground state conformational dynamics<sup>(3)</sup> and excited state potential energy surfaces,<sup>(4)</sup> and synthetic nucleotide chemistry.<sup>(5)</sup> The *cis-syn* cyclobutane dimer formed in a [2 + 2] cycloaddition reaction between adjacent thymines is the major product of irradiation of duplex DNA, accompanied in some cases by lesser amounts of the 6-4 adduct, which is thought to arise from a Paterno-Büchi cycloaddition followed by thermal ring opening of the oxetane intermediate. Both the efficiency of product formation and the product ratio in duplex DNA are dependent upon the base sequence, lower yields being observed for flanking purines vs. pyrimidines. Dimerization efficiency has also been related to conformational flexibility. Recently attention has focused on the importance of ground state conformational populations in determining T-T dimerization efficiency.

## EXPERIMENTAL

Oligonucleotide conjugates **1-10** and single strand sequences **11a,b** (Chart 1) were prepared using standard phosphoramidite chemistry, purified using reverse phase HPLC, and characterized by MALDI-TOF mass spectrometry.

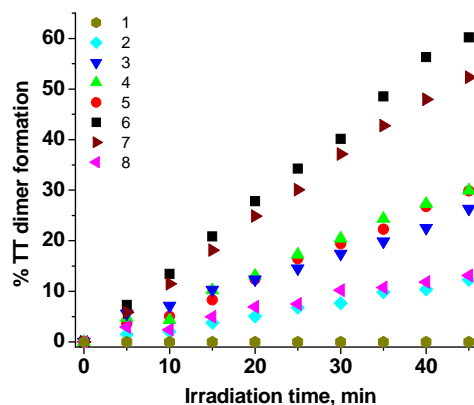


Solutions containing ca. 1-1.2  $\mu\text{M}$  hairpin in standard buffer were irradiated with monochromatic 280 nm light at 10 °C in 1 cm pathlength quartz cuvettes. The progress of irradiation was monitored at 260 nm by high temperature HPLC with a column temperature of 60 °C.<sup>(5)</sup> The use of high temperature HPLC to achieve thermal rather than chemical denaturation permits analysis of small aliquots of irradiated solutions with excellent resolution.

## RESULTS

With the objective of understanding the context dependence

in the efficiency of photodimerisation in isolated thymine thymine (TT) steps in DNA, we have carried out careful investigation on the formation of thymine dimer by varying TT pair as a function of position in DNA hairpin sequences **1-10** containing C12 linker and single strand sequences **11a-11b**. Our results for hairpins **1-5** and single strands **11a,b** are consistent with previous reports that the 2+2 dimer is the major product obtained from TT steps in either duplex and single strand DNA, but is not formed from alternating AT sequences. Unprecedented are the observations of (a) identical reactivity for the interior TT steps in **3-5**, (b) diminished dimerization efficiency for TT steps at the 5'-3' hairpin terminus of **2** and the single and double T and TT overhangs in **8** and **9**, as well as in the single strand **11a**, (c) enhanced 2+2 dimerization at the site of a single T bulge in **7**, and (d) formation of both 2+2 and 6-4 products adjacent to the alkane linker in **6**. These observations can be understood in terms of the topochemical principles of Fischer for solid state photodimerization as applied by Kohler and others to T-T dimerization in DNA. That is, dimerization occurs only in a sub-set of ground state conformations in which the adjacent thymines have their chromophores properly aligned so that bonding can compete with ultrafast non-radiative decay or energy transfer.<sup>(6)</sup>



## REFERENCES

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